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NUTRITION REVIEWS

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NUTRITION REVIEWS

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ADAPTATION TO LACTOSE

Lactose is the only carbohydrate found in substantial amount in milk. It alone accounts for approximately half the total solids in this, the first food of all mammals. The ability of young animals to digest this sugar, therefore, is an absolute necessity for survival.

It has been known for many years that the digestion of lactose starts with hydrolysis of the disaccharide to its component monosaccharides glucose and galactose, which are then absorbed and utilized. This hydrolysis is catalyzed by lactase, an enzyme present in the small-intestinal mucosa.

Lactase, since it cleaves the β-galactosidic linkage in lactose, is a β-galactosidase. However, not all β-galactosidases attack lactose. There is present in most animal tissues, including liver, kidney and spleen, and also the intestinal mucosa, a β-galactosidase which does not break down lactose at an appreciable rate (N. S. C. Heilskov, *Studies on Animal Lactase*, Munksgaard, Denmark, 1956). This could explain why, although β-galactosidase activity has been found outside the digestive tract, lactose injected into the bloodstream is not metabolized, but is excreted unchanged. The natural substrates of this more generally distributed β-galactosidase are unknown. For those studying lactose metabolism, this enzyme assumes an added importance in that attempts to use chromogenic substrates (e.g., orthonitrophenyl β-D-galactoside) as substrates for lactose must account for non-lactase-β-galactosidase activity.

In the last decade of the nineteenth and first decade of the twentieth century, a number of physiologists in this country and abroad were interested in the digestion of lactose. Their studies suggested that the major location of lactose digestion was in the small intestine and that the intestines of young mammals were more active in hydrolyzing lactose than those of older ones. E. Fischer and W. Niebel (*Sitzber. Akad. Wiss. Berlin, p. 73 (1896)*) suggested that the feeding of young animals on lactose (milk) might be responsible for their higher "lactose ferment" activity. That is, that lactase production by the intestine is an adaptation to lactose.

A controversy soon arose between those who believed in the adaptation theory and those who felt that there was no experimental evidence to support the hypothesis. At one time Pavlov, himself, suggested that pancreatic enzymes were produced in response to the type of food ingested (B. P. Babkin, *Secretory Mechanism of the Digestive Glands*, Hoeber, New York, 2nd ed. 1950). There were claims and counterclaims made, supported by various experimental data, but without the concept of pH, and adequate awareness of the dangers of bacterial contamination, it was not possible to achieve clearcut answers.

R. H. A. Plimmer (*J. Physiol.* **35,** *20 (1907)*) in a review of previous work on the subject, and based on much work of his own, using better assay conditions, concluded that lactose adaptation in the pancreas does not occur. He went on to look for evidence of lactose adaptation in the intestine of rat, guinea pig, rabbit, pig and chicken. He found none, and concluded that neither the pancreas nor the intestine can be made to adapt to lactose. Recent work substantiates Plimmer's observation that the feeding of lactose to weaning animals does not keep the lactase activity at the high level present in the very young (N. S. C. Heilskov, *Acta physiol. Scandinav.* **24,** *84 (1951)*; J. E. Fischer, *Am. J. Physiol.* **188,** *49 (1957)*).

In order to gain perspective on the problems of adaptation, let us, at this point, digress for a moment from studies in higher animals and consider briefly what is known

about the adaptation of bacterial populations. Much work has been done on the means by which a bacterial population succeeds in growing under particular conditions, such as ion concentration, pH, aeration, sources of carbon, and nitrogen.

For example, *Escherichia coli* can use lactose as its only source of carbon and energy, if allowed to "adapt." Basically, two types of adaptation are possible: either there are cells in the population which, as is, are capable of growing on the lactose, and these cells are selected for, and grow up rapidly, or the presence of lactose evokes the production of an enzyme (or enzymes) which then enables any cell so affected to grow on the lactose. In the former case, while the total enzyme activity of the population will go up, the amount of enzyme in a given cell will be the same as the amount of enzyme in the cell originally selected for, whereas in the latter case the enzyme content per cell will increase. This situation, where a compound evokes enzyme production, is referred to as induced enzyme synthesis.

Many investigators (J. Lederberg, *J. Bacteriol.* **60,** *381 (1950)*; J. Monod and M. Cohn, *Advances in Enzymol.* **13,** *67 (1952)*; M. Cohn, *Bacteriol. Rev.* **21,** *140 1957*)) have studied in great detail the induced synthesis of *E. coli* β-galactosidase (lactase). They have shown that the enzyme can hydrolyze a variety of alkyl and aryl β-galactosides, and that most of these compounds can serve, not only as a source of sugar, but as inducers for β-galactosidase. Further, Monod has shown that exposure of the cells not only to hydrolyzable galactosides, but to non-hydrolyzed thiogalactosides, can, *under the proper conditions*, result in the synthesis by the cells, of β-galactosidase. It is the necessary conditions which are of interest here, since certain of them may give clues to understanding adaptation in animals.

Using thiogalactosides, it is possible to get induction of galactosidase while the cells are contentedly growing on another sugar. Thus induction of galactosidase in strains which will not grow on lactose can be studied, and from the results much can be learned of the mechanism and requirements of induction. There are two types of such strains: in one there is an hereditary defect which renders the cell incapable of producing galactosidase, and in the other, an hereditary defect which keeps the cell from producing an enzyme-like system which is responsible for facilitating the entrance of galactosides into the cell. If inducer concentrations high enough to overcome the natural permeability barrier of the cell are used with the latter strain, induction occurs (L. A. Herzenberg, *Biochim. Biophys. Acta* **31,** 525 (1959)). However, with this impermeable (cryptic) strain, lactose itself, regardless of concentration, will not induce galactosidase, an observation as yet to be satisfactorily explained.

Thus, genetic makeup is a key factor in allowing induction. Bacteria must be genetically competent of responding to inducer. Permeability of the cell to inducer is important, as well as the choice of inducer. Age and state of nutrition of the cell too, among other factors, influence the cell's inducibility.

Bearing in mind the necessary conditions for adaptation in bacterial populations, let us re-examine Plimmer's conclusion that "neither the pancreas nor the intestines of animals can be made to adapt themselves to any particular diet" (R. H. A. Plimmer, *J. Physiol.* **35,** *20 (1907) p. 30*).

Several questions are immediately suggested: 1) Is lactose an inducer of lactase in animals, or is there perhaps another inducer (a β-galactoside, a hormone?) for lactase in animals? 2) Are cells of the intestinal mucosa of weanlings and adults "genetically" competent to respond to lactose (or any other inducer) and produce lactase, or has the process of differentiation and development resulted in the loss of a characteristic perhaps originally present in animal cells? 3) Since dietary lactose never reaches the bloodstream in a significant amount in adult animals, is it possible that it never reaches a site where it can act effectively as an inducer? Even if this site were in the intestinal mucosa, it is possible to imagine that the

lactose is broken down before it either can penetrate the intestinal mucosa sufficiently to induce or build up a sufficient concentration in the cell to bring about induction.

The questions raised suggest that it might be better to look for induction in very young animals, where the intestine might contain cells which have not yet lost the ability to adapt, and where the intestinal wall has not yet thickened and is still somewhat permeable to most substances.

The pattern of appearance and increase of lactase in fetal and newborn animals could be interpreted as an enzyme induction phenomenon. Recent work (N. S. C. Heilskov, *Studies on Animal Lactase*, Munksgaard, Denmark (1956)) has shown that intestinal lactase increases during the latter part of gestation and reaches a maximum specific activity a short time after birth. It is possible that fetal and infant intestinal mucosa cells, still genetically competent to produce lactase, are induced to form lactase by exposure to some substance present late in fetal life and early in lactation. This "substance" could be lactose, liberated into the mother's bloodstream and entering the fetus via the placenta, and later possibly entering the infant's bloodstream due to a more permeable intestinal wall. It might also be a hormone such as prolactin, whose presence coincides with the period of increased synthesis of the enzyme. Whatever the details, there is a definite possibility that lactase can be induced in mammals.

Adaptation by animals to lactose, however, is not limited to induction of lactase. Rats fed a diet rich in lactose develop diarrhea, but after continued administration of the sugar, the animals adapt to the unnatural diet conditions and the diarrhea subsides (L. K. Riggs and A. Beaty, *J. Dairy Sci.* **30,** *939 (1947)*).

Fischer and her collaborators have shown that the weight of intestinal mucosa of lactose-adapted rats has increased about 50 per cent, giving the adapted individuals larger amounts of lactase without increasing the amount of lactase per intestinal mucosa cell (J. E. Fischer, *Am. J. Physiol.* **188,** *49 (1957)*). In addition, these animals develop a larger cecum (J. V. Lawrence, J. E. Fischer, T. S. Sutton and H. H. Weiser, *Ohio J. of Sci.* **56,** *87 (1956)*). It was also shown that lactose disappeared from the intestine about 50 per cent faster in adapted animals (J. E. Fischer and T. S. Sutton, *J. Dairy Sci.* **36,** *7 (1953)*), and presumably was hydrolyzed and utilized by the animals. There have been other studies, not as fully analyzed as Fischer's case where, after an initial diarrhea, various animals have been shown to adapt to the lactose diet (H. S. Mitchell and W. M. Dodge, Jr., *J. Nutrition* **9,** *37 (1935)*; E. O. Whittier, C. A. Cary and N. R. Ellis, *Ibid.* p. 521).

The animal, then, has two mechanisms for dealing with lactose. The infant, who depends on lactose for nourishment, is provided with a mechanism which, even before birth, has begun to raise the lactase level in the cells of the intestinal mucosa. This level, generally highest soon after birth, falls slowly (probably due to a decreasing rate of synthesis of lactase) until it reaches a basal level after weaning. From this time on, the basal level prevails, and in order to handle large amounts of lactose, the animal is forced to resort to a second mechanism, that of increasing the mass of the intestinal mucosa in order to have sufficient lactase to deal with the increased dietary burden.

An understanding of what triggers lactase synthesis towards the end of fetal life and the factors which maintain a constant lactase level in the adult intestinal mucosa even though the animal may need more lactase, would be of theoretical significance and would certainly rapidly find practical applications.

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NITROGEN RETENTION DURING THE FIRST SIX MONTHS OF LIFE

The lack of a recommended dietary allowance for protein for young infants by the Food and Nutrition Board (*Recommended Dietary Allowances, National Academy of Sciences-National Research Council, publication* **589** (*1958*)) reflects both the disagreement between authorities and paucity of basic information. Recent studies at the University of Iowa have added to our knowledge concerning the nitrogen balance, growth patterns, and some serum constituents in infants fed human milk and a formula derived from cow's milk containing an intermediate amount of protein.

The techniques used in these studies were described in detail by S. J. Foman, L. N. Thomas, R. L. Jensen and C. D. May (*Pediatrics* **22,** *94* (*1958*)). They were carried out in a metabolic ward which has been in use for many years. The majority of the subjects were infants of students in the university, of resident physicians, of unwed mothers or of women with tuberculosis. Each infant was fed the largest amount of milk that would be entirely consumed. Feedings were offered at four-hour intervals. The infants were fed only milk and supplementary vitamins.

Approximately every two weeks between birth and six months of age, a three-day balance was carried out. Aliquots of the milk mixture from every 24 hour feeding were prepared for analysis. Urine and feces were collected separately from boys, but no attempt was made to collect them separately from girls. Nitrogen determinations in food aliquots as well as the excreta consisted of micro Kjeldahl digestion modified after the method of D. D. Van Slyke and B. H. Kugel (*J. Biol. Chem.* **102,** *189* (*1933*)), with determination of nitrogen concentration in the diluted digest according to the method of E. J. Conway and E. O. O'Malley (*Biochem. J.* **36,** *655* (*1942*)).

Data on replicate determinations of nitrogen on specimens of milk, feces and urine were presented which show very little variability. Thirteen pairs of consecutive three-day metabolic balance studies on five infants were presented showing the degree of variability which can be expected in such studies. Their statistical considerations on the reproducibility of nitrogen balance studies have not yet been published.

Nine full-term infants were fed human milk during the first six months of life (Foman and May, *Pediatrics* **22,** *101* (*1958*)). Six of these received human milk as the sole food during nearly all of this period. Three infants alternated between human milk and a formula prepared from cow's milk. Daily collections of milk from individual donors were pooled, pasteurized and frozen. The mean concentration of nitrogen in the collected milk varied from 1.9 to 1.6 g. per liter during the course of the study.

The growth curves of the nine infants on Iowa growth charts were presented graphically. In all cases the increase in weight and length paralleled standards. It should be pointed out that none of the infants exceeded the fiftieth percentile for weight at the age of six months, although their lengths appeared to have a normal distribution. The concentration of hemoglobin in the blood between three and five months of age generally ranged between 9 g. and 10 g. as compared with normal values of 12.2 ± 2.0 g. per cent. The total nitrogen in the serum averaged 8.4 ± 1.2 g. per l., which would be equivalent to an average of 5.2 g. protein per cent. The mean concentration of urea nitrogen was 6.3 mg. per cent.

The data from 74 metabolic balance studies are presented graphically. The data for the first 45 days and the last 45 days of the total study were most representative. The volume of intake decreased from 218 ± 22 initially to 140 ± 10 ml. per kg. per day at four and one-half to six months. The resulting nitrogen intakes were 383 ± 45 mg. per kg. per day during the initial 45

days and 240 ± 27 mg. per kg. per day during the last period. The intake of nitrogen was correlated positively with the nitrogen retention, and the regression line had a slope of +0.67 and a standard error of the estimate of ±22.3 mg. per kg. per day. The ratio of gain in weight to retention of nitrogen remained quite constant, having a mean of 42.1 ± 11.5.

These data indicate a caloric and protein intake for the first six weeks of life of 147 calories and 2.4 g. of protein per kg. per day; for four and one-half to six months of age, 94 calories and 1.5 g. of protein per kg. per day. The mean retention of nitrogen at these same ages decreased from 180 to 47 mg. per kg. per day at six months.

In discussing the results, Foman and May point out that there is no reason at the present time not to accept the performance of the human infant fed nothing but breast milk during the first six months of life as a standard reference for normal infants. Their data represent the metabolic performance of infants fed pasteurized human milk. The fact that the nitrogen retention, serum protein and serum urea nitrogen concentrations appeared to be somewhat lower than values generally accepted as normal can be considered only to be descriptive of the infant fed human milk. The removal of blood for chemical determinations was accepted as the explanation of the low hemoglobin concentrations in this group of infants who did not receive supplemental iron.

The mean gain in weight for each gram of nitrogen retained was 42.1 g., which is considerably greater than the 27 g. calculated from the data of P. C. Jeans *et al.* (*J. Pediat.* **8,** *403* (*1936*)) on infants fed higher protein intakes derived from cow's milk. This ratio is important in that it measures, in part, the difference in body composition which must exist between breast fed infants and those fed greater amounts of protein.

In order to translate the data obtained from infants fed pasteurized human milk it was necessary to show that fresh human milk would give similar responses. Foman, Thomas, and May (*Pediatrics* **22,** *935* (*1958*)) performed 29 nitrogen balance studies on ten normal full term infants fed fresh human milk either from the breast or by bottle. These infants were brought into the metabolic ward from their homes for these studies; the milk supply came largely from women relatively late in lactation and had a somewhat lower nitrogen content than in the previous study. Consequently, the nitrogen intake of these infants was somewhat reduced.

The retention of nitrogen with respect to intake, however, appeared to be quite comparable to that of the infants fed pasteurized human milk. As far as nutritional value was concerned there was no apparent difference between processed human milk (pasteurized and frozen) and the fresh product. Consequently, the studies of infants fed pasteurized human milk can be considered representative of infants fed breast milk under natural circumstances.

Since the nitrogen retention of infants fed human milk *ad libitum* was less than that of those reported by Jeans *et al.* (*loc. cit.*) for infants fed formulas of cow's milk containing considerably higher concentrations of protein, Foman and May (*Pediatrics* **22,** *1134* (*1958*)) investigated the nitrogen balance of infants fed a preparation of cow's milk which supplied an intermediate intake of protein. They used a commercial formula supplying 1.7 g. of protein, 6.6 g. of lactose, and 3.3 g. of fat per cent. Seventy-one nitrogen balance studies were performed on 12 infants. Nine of these infants received this formula as their sole food intake during the first six months of life, and three alternated between this formula and human milk. In the present study the formula was fed *ad libitum*, in exactly the same manner as the processed human milk.

The growth of the infants proceeded along normal patterns but generally fell at, or below, the fiftieth percentiles of the Iowa

growth charts, as did the breast-fed infants. The mean concentration of total protein in the serum was 5.3 g. per cent, which is quite comparable to that of the breast-fed infants. The urea nitrogen was somewhat higher at 8.9 mg. per cent. The mean volume of intake was somewhat less than that of breast-fed infants at 198 ml. per kg. per day during the first one and one-half months of life but similar during the period between four and one-half and six months. The protein intakes were higher, being equivalent to 3.3 g. of protein per kg. per day during the first 1½ months and 2.5 g. per kg. per day during last six weeks of the study.

The retentions of nitrogen were 211 and 102 mg. per kg. per day in these same periods. These nitrogen retentions were greater than those observed on infants fed human milk. Exact comparisons between nitrogen intake and nitrogen retention were almost impossible because of the age differences between the two groups which received a similar nitrogen intake. For each gram of nitrogen retained, however, the mean gain in body weight was 33 g., which was only slightly greater than the value of 27 g. found by Jeans.

These descriptive studies have added greatly to our fund of information concerning nitrogen retention in infants. More knowledge concerning the significance of the differences in body composition among infants fed various types of milk will have to be obtained before all the implications of these differences really can be understood.

ALDOSTERONE IN SODIUM HOMEOSTASIS IN MAN

The significance of aldosterone as the adrenal steroid predominantly concerned with the regulation of renal sodium excretion has been clearly established by the many investigators who have contributed to this field since the isolation of the hormone in 1952. Much of this work has been reviewed previously (*Nutrition Reviews* **11,** *218 (1953)*; **12,** *280 (1954)*; **13,** *281 (1955)*; **15,** *345 (1957)*).

One of the basic observations in this field has been the evidence that restriction of dietary sodium results in morphologic alterations in the adrenal and increased amounts of aldosterone in the urine. The specific stimulus for increased aldosterone secretion in man and the detailed relationship between dietary salt and aldosterone secretion are questions of interest for nutritionists.

F. C. Barter (*Metabolism* **5,** *369 (1956)*) has made some very challenging hypotheses concerning aldosterone secretion. He has summarized clinical investigations which suggest that the release of aldosterone by the adrenal is controlled by some function of the extracellular fluid volume. Under controlled conditions, major alteration in aldosterone excretion could be induced by expanding or contracting the extracellular space without alteration in total body sodium. The level of serum potassium which has been ascribed a major role in aldosterone regulation by Laragh and Stoerk (*Nutrition Reviews* **13,** *282 (1955)*) was relegated to secondary importance by Barter.

Recent observations from the clinic of Dr. Thorn in Boston suggest that the concepts of regulation of aldosterone secretion may have to be revised. The adrenal cortical response to altered dietary sodium intake has been described in two papers.

In the first, J. Crabbé, W. J. Reddy, E. J. Ross and G. W. Thorn (*J. Clin. Endocrinol.* **18,** *1147 (1958)*) have examined the total adrenal response to sodium restriction. The abrupt restriction of dietary sodium from 200 mEq per day to 8 mEq per day was associated with an increase in aldosterone in repeated studies in one individual. The excretion of the active corticoid hormones (cortisol and cortisone) determined

chromatographically was unchanged by the dietary shift. The 17-hydroxycorticosteroids measured by the Porter Silber method similarly were not related to sodium intake. It is unlikely that sodium restriction by itself altered the excretion of corticosteroids because of the pattern of corticosteroid excretion in a patient receiving a constant dose of cortisone (50 mg. per day) was uninfluenced by sodium restriction.

In the absence of aldosterone, even relatively large doses of corticosteroids did not correct the inability to conserve sodium in Addison's disease. These various observations confirm the findings of others that the secretion of aldosterone and corticosteroid hormones are independently regulated.

The second paper of this series is an attempt to correlate aldosterone excretion with dietary sodium intake (Crabbé, Ross, and Thorn, *J. Clin. Endocrinol.* **18,** *1159 (1958)*). The reduction of dietary sodium to 8 mEq per day in five normal subjects resulted in an increase in aldosterone excretion. It was noteworthy, however, that the aldosterone did not continue to rise during the period of sodium restriction but reached a peak, in most subjects, usually after three to seven days with a subsequent major decline. In one subject the aldosterone excretion was only two micrograms per day at the end of the ten day period of observation. This value, which is below the normal mean excretion, occurred at a time when renal sodium conservation was nearly maximal. The aldosterone excretion associated with acute dietary sodium restriction was increased by preliminary treatment with acetazolamide.

The sodium intake was reduced in stepwise manner every six days from 210 to 70, 25 and 8 mEq per day in two subjects. The first subject had responded to acute sodium deprivation with only a transitory increase in urine aldosterone. Little augmentation of aldosterone excretion also occurred with stepwise reduction of sodium intake. Actually the urinary levels were higher on the 25 mEq sodium diet than on the 8 mEq diet.

The response of the second subject was different in that greater rises in aldosterone excretion occurred, but the correlation was poor of the aldosterone excretion with the degree of sodium restriction or with renal excretion of sodium.

In all the varied situations the resumption of normal sodium in the diet was attended by a decrease in urinary aldosterone excretion which occurred promptly. This adjustment required only one or two days and occurred at a time of nearly maximum sodium retention.

The general correlation of dietary salt and urinary aldosterone excretion is confirmed by these studies but some very obvious peculiarities are evident. It was difficult to correlate the degree of aldosterone excretion with either the depletion of extracellular sodium or the magnitudes of the urinary sodium. Individual variation in the aldosterone excretion in response to the experimental manipulations were most conspicuous. The authors would like to attribute the failure to find a clear relationship between aldosterone excretion and sodium conservation to independent renal mechanisms of salt conservation. The possible nature of these mechanisms was not elaborated upon, but may have to do with the quantity of sodium presented to the tubule for reabsorption.

It is implicit in the above discussion that existing measurements of urinary aldosterone are indicative of the actual production of aldosterone by the adrenal and are proportional to the active level of aldosterone in the blood. Even excluding the matter of analytic errors, it is quite possible that the peculiarities in aldosterone excretion may represent alterations in the renal handling of aldosterone. The limited accuracy and the tedious nature of the measurement of aldosterone have retarded solution of such problems. The development of a double isotope dilution method for the measurement of aldosterone (B. Klimen and R. E. Peterson, *Fed. Proc.* **17,** *255 (1958)*) in blood as well

as urine with accuracy, if not simplicity, will greatly assist in answering the problems raised by the studies of Crabbé and coworkers.

In summary, it may be said that additional evidence has been presented for the independence of aldosterone and corticosteroid excretion and the dependence of normal salt conservation on aldosterone. The relationship between the degree of salt retention and the amount of aldosterone excretion may not be as simple as once thought. There also appears to be a considerable person to person variation in the secretion of aldosterone when sodium restriction is maximal.

ANABOLIC STEROIDS AND UREMIA

The end products of metabolism of carbohydrate and fat are lost mainly in the breath as carbon dioxide, but those resulting from the degradation of protein, urea and other nitrogenous substances, potassium and fixed acids are excreted in the urine. Thus, in patients with severe kidney damage, these products accumulate in the body, giving rise to the signs and symptoms of uremia.

In the usual method of treating patients with uremia, giving excessive amounts of water and electrolytes is avoided and all methods of reducing protein metabolism to a minimum are employed. However, the level of protein metabolism in the uremic patient being maintained solely on glucose and electrolytes is still above that of normal individuals on a similar regimen, considering the rate of increase of urea or nonprotein nitrogen in the blood and body fluids.

In seeking some method of reducing the rate of urea formation, it was noted that testosterone and some of its derivatives have been known for some time to exert an anabolic effect; that is, they tend to produce a positive nitrogen balance in both undernourished individuals and post-traumatized patients who are given a nitrogen-containing regimen. Synthetic derivatives of testosterone, 17-α-ethyl-19-nortestosterone and 19 - nortestosterone - 17β - phenylpropionate, have been developed which have anabolic activity and decreased androgenic effects when tested in animals.

Recently, two preliminary reports appeared in which attempts were made to reduce the rate of protein degradation in patients with uremia by giving them the anabolic steroids in addition to the usual regimen. Thus, by reducing the rate of protein degradation, it was thought that the toxic effects of the uremia could be reduced and time would be made available to allow the kidney to recuperate where possible. B. H. McCracken and F. M. Parsons (*Lancet* **II,** *885 (1958)*) have studied the rate of urea production in 13 patients with acute renal failure and compared it with the urea production in five healthy adults. Of the 13 patients, six were obstetrical patients and seven had renal failure not related to obstetrical problems. All the patients with renal failure were maintained orally on a total fluid intake of about 400 ml. per day and were receiving approximately 400 calories derived from 100 g. of glucose. Vitamin supplements were also given. The five healthy individuals were maintained on a constant diet which contained 120 g. of glucose and 2 g. of nitrogen. This regimen supplied 600 calories per day.

Urea production was used as an index of protein catabolism in these studies. The amount of urea excreted in the urine, if any, in the uremic patients was added to the amount which was calculated to accumulate in the body fluids. This amount was determined from the daily increase in urea nitrogen of the blood multiplied by the total body water. The latter was estimated from

the body weight. The first four days the patients received the anabolic steroid in addition to the same nutritional regimen as used previously. The usual dose of 17-α-ethyl-19-nortestosterone employed in these studies was 80 mg. per day given in divided doses.

The mean daily urea production for the six obstetrical patients during the control period was 9.1, 6.4, 4.1, 8.4, 6.6 and 4.3 g. After receiving the anabolic steroid, the daily urea production fell to 1.8, 2.1, 0.2, 4.1, 2.3 and 1.9 g., respectively, for the same patients. On the average, a 70 per cent reduction in the rate of protein degradation occurred in the patients getting the anabolic steroid, if the rate of urea formation were used as an index.

On the other hand, little or no change was noted in non-obstetrical patients with renal failure (glomerular disease, diodone reaction, ureteral obstruction and extensive burn) who were given the anabolic steroid. The maximum change noted in this group was in a patient with glomerular disease who had a mean control urea production of 5.0 g. per day, which fell to 3.0 after giving the steroid.

A patient with extensive burns had an average daily urea production of 16.1 g. before he was given the steroid, and 16.4 g. afterward. Also, only small and inconsistent changes were noted in the average rate of urea production in the healthy individuals when they received the anabolic steroid in addition to the low calorie and protein diet.

In an accompanying note, S. Gjørp and J. H. Thaysen (*Lancet* **II,** *889 (1958)*) have also reported the beneficial results of anabolic steroids on ten patients with severe subacute renal failure. The results obtained from one individual are given. On a nitrogen intake ranging from 0 to 3 g. per day, the non-protein and urea nitrogen in the blood rose to 200 and 150 mg. per 100 ml., respectively. Upon giving the anabolic steroid at a dose level of 40 mg. per day, a sharp decline in the nitrogenous constituents of the blood was noted in spite of the patient receiving a nitrogen intake as high as 12 g. on some days.

At the end of eight days of steroid therapy, the non-protein and urea nitrogen levels of the blood fell to about 150 and 100 mg. per 100 ml., respectively. During this period the nitrogen output fluctuated between 8 and 4 g. per day. For the next six days the steroid therapy was withheld and nitrogenous constituents of the blood rose. On repeating the steroid therapy, the non-protein nitrogen and urea nitrogen levels of the blood fell. The blood levels of urea and non-protein nitrogen remained relatively constant at about 125 and 150 mg. per 100 ml. for about 24 days while the patient was receiving the anabolic steroid. In all of the ten patients with severe chronic renal failure, the anabolic steroids caused an increase in appetite, an improvement of the clinical condition, an increase in lean body mass as indicated by balance studies, and an increase in body weight. In no case did an increase in the protein intake of the patient cause an increase in the biochemical indices of uremia when the anabolic steroid was used. These investigators believe that the use of the anabolic steroid might break the cycle of progressive uremic intoxication and the approaching cachexia in patients with renal failure. While there is no evidence that the anabolic steroids actually aid the regeneration of the kidney in renal failure, their use allows the patient more time to permit any reparative process to take place.

Summary: The employment of anabolic steroids in patients with renal failure has the advantage of hindering the breakdown of body tissues and this, in turn, reduces the amount of nitrogenous waste products in the blood (*e.g.* lowers the blood non-protein nitrogen and urea levels). Also, the employment of such steroids may allow the patient to receive a diet higher in protein without increasing the biochemical and clinical signs and symptoms of uremia.

REQUIREMENTS FOR SULFUR AMINO ACIDS

Observations on diets self-selected by women have suggested that methionine is the amino acid most likely to be deficient under conditions of normal food intake. The proposed minimum requirement, until recently, has been 1.1 g. per day, based on the needs of young men maintained on an artificial diet which contained no cystine (W. C. Rose, M. J. Coon, H. B. Lockhart and G. F. Lambert, *J. Biol. Chem.* **215,** *101 (1955)*). However, the results of several other studies have indicated that in the presence of cystine the minimal methionine requirement is much less (W. C. Rose and R. L. Wixom, *J. Biol. Chem.* **216,** *763 (1955)*).

In order to achieve some further elucidation of this problem, M. S. Reynolds, D. L. Steel, E. M. Jones and C. A. Bauman (*J. Nutrition* **64,** *99 (1958)*) have conducted studies of nitrogen balance on 21 women fed semi-artificial diets in which levels of methionine and cystine were manipulated.

The subjects were women students or staff-members who maintained their usual academic pursuits throughout the study. They were fed a diet of natural foods for eight to 12 days in order to accustom them to experimental routines and to the level of nitrogen intake employed throughout the study. During a transition period of three to four days, the natural foods were gradually replaced by a semi-artificial diet, including various amino acids, which was then fed throughout the remainder of the study. In some cases methionine was omitted from the amino acid mixtures both in order to demonstrate the need for this amino acid in the subjects participating in the studies and to demonstrate that the natural foods in the basal diet did not furnish enough methionine to meet the requirements by the subjects.

The semi-artificial diet consisted of a few low-protein foods such as canned fruits, butter oil, cornstarch, sucrose and vegetable oils, as well as amino acids, diammonium citrate, purified hemicellulose and mineral and vitamin supplements, and supplied from 10 to 11 g. of nitrogen per day.

When 500 mg. of cystine was supplied in conjunction with various levels of methionine, seven subjects receiving 30 or 80 mg. methionine showed nitrogen losses ranging from 0.45 to 1.04 g. per day. Increasing the methionine intake of these seven subjects to 150 to 180 mg. per day resulted in nitrogen storage for four subjects and only small losses for the other three subjects. In two subjects, following eight days of the above intake, cystine was removed for a period of seven days leaving only some 10 mg. furnished by natural foods. Nitrogen losses were observed until the cystine was replaced.

With only the 10 mg. of cystine furnished by the natural foods of the diet, intakes of methionine ranging from 30 to 150 mg. resulted in nitrogen loss by all four subjects given these amounts. Of 11 other subjects who were fed 290 mg. of methionine, only three stored nitrogen, while the losses in the remaining eight subjects ranged from 0.01 to 1.19 g. Two subjects were given 500 and 850 mg. of methionine, respectively. Their nitrogen balances were +0.02 and −0.14. The authors point out that if one accepts the "zone of equilibrium" proposed by R. M. Leverton *et al.* (*J. Nutrition* **58,** *59 (1956)*) as nitrogen balance, the requirement for nine of 13 subjects was met with 10 mg. of cystine and 290 mg. of methionine, or a total of 300 mg. of the sulfur-containing amino acids. However, if strict adherence to positive values in the nitrogen retention column is maintained as a criterion for nitrogen balance, more cystine is required.

The latter result prompted a comparison of the effectiveness of varying amounts of cystine added to a constant diet of 290 mg. of methionine. An intake of cystine amounting to 260 mg. permitted nitrogen storage ranging from 0.06 to 0.83 g. in six subjects. When cystine was reduced in three of these subjects to 140 mg. per day, one of them lost

1.5 g. of nitrogen per day. Further reduction of cystine to 10 mg. resulted in nitrogen loss in five of the six women.

Thus, according to the Leverton "zone of equilibrium" the requirement for sulfur-containing amino acids in women may be as low as 300 mg. when a large proportion of the total is in the form of methionine, while 150 to 180 mg. of methionine represents the borderline of requirement when generous amounts of cystine are supplied. If, however, the Rose criterion of positive storage is applied, 240 to 290 mg. of methionine with 500 mg. of cystine meets the requirement for most subjects.

These amounts are of the same magnitude as those reported by M. Swendseid, I. Williams and M. S. Dunn (*J. Nutrition* **58,** *495 (1956)*), who found a total requirement of 350 mg. or less of total sulfur-containing amino acids for five women, 450 mg. for two others, and 550 mg. for another when the diet supplied 7 to 8 g. of nitrogen as peanut protein.

The rather great variability in individual requirements of sulfur-containing amino acids may be due in part to individual variations in rates of protein synthesis and/or tissue growth and repair. Sulfur-containing amino acids are known to be in demand by growing animals and in sites of wound healing. Additional studies are required to elucidate more fully the effects of these factors.

ASCORBIC ACID IN SERUM

Several studies have indicated that dehydroascorbic acid can be reduced in the tissues of man. Ingestion of orange juice treated with norite or iodine to oxidize naturally occurring ascorbic acid is followed by excretion of as much ascorbic acid in the urine as when untreated orange juice is given (D. N. Todhunter, T. McMillan and D. A. Ehmke, *J. Nutrition* **42,** *297 (1950)*). Similar increases of ascorbic acid have been found in the plasma following the ingestion of crystalline dehydroascorbic acid (H. Borsook, *et al., J. Biol. Chem.* **117,** *237 (1937)*).

Studies comparing the concentrations of the two forms of the vitamin in the serum after oral administration of both ascorbic acid and dehydroascorbic acid have been carried out by H. Linkswiler (*J. Nutrition* **64,** *43 (1958)*). These experiments were designed to measure the extent and rate of reduction of dehydroascorbic acid by determining the ratios of ascorbic acid to ascorbic acid plus dehydroascorbic acid in serum before and at various intervals following ingestion of the two substances.

Seven women, ranging in age from 25 to 40 years served as subjects. A fasting blood sample was taken, after which the subjects were given either dehydroascorbic acid (50, 150, 300 or 500 mg.) or ascorbic acid (150 or 500 mg.). Blood was analyzed for total ascorbic acid 1, 1.5, 3, 5 and occasionally 24 hours after the ingestion of vitamin. Blood samples from fasting individuals contained from 0.47 to 1.7 mg./100 ml. serum and averaged 1.28 mg. The proportion of the total ascorbic acid present in the dehydro form ranged from 7 to 41 per cent (mean, 21 per cent). In 22 samples dehydroascorbic acid accounted for 16 to 26 per cent of the total ascorbic acid concentration; in five samples the values were 10 per cent or less and in seven samples 30 per cent or more. Serum containing a high concentration of ascorbic acid tended to contain a correspondingly high concentration of dehydroascorbic acid.

Following ingestion of either form of the vitamin there was a rapid increase in both ascorbic acid and dehydroascorbic acid in serum, the peak in concentration occurring at 1.5 hours. The time required for maximum concentration was apparently unaffected by the form of the vitamin given. A significant

decrease in blood levels occurred between 1.5 and 3 hours after ingestion. Three hours after an intake of 50 mg. of dehydroascorbic acid, the concentration of the vitamin had returned to the original fasting level. Intakes of 150 mg. or more resulted in concentrations which persisted above the fasting level for five hours or more but which were restored to the original level within 24 hours.

The increments of increase in total ascorbic acid (ascorbic acid plus dehydro-) after ingestion of 50, 150, 300, or 500 mg. dehydroascorbic acid were 0.19, 0.54, 0.81 and 1.02 mg./100 ml. while corresponding values for ingested ascorbic acid were 0.13, 0.36, 0.60 and 0.98 mg. After ingestion of 150 or 500 mg. ascorbic acid the increases in total ascorbic acid were 0.44 and 11.7 mg./100 ml. and in ascorbic acid were 0.44 and 0.98 mg., respectively. Variance analysis showed that equal intakes of either form of the vitamin resulted in comparable increases in both dehydroascorbic acid and ascorbic acid. This is in agreement with the results of B. E. Clayton, *et al.*, (*Biochem. J.* **58,** *542* (*1954*)) who reported that in human subjects oral dehydroascorbic acid behaved quantitatively like ascorbic acid.

There was an absolute increase in the concentration of dehydroascorbic acid in serum 1.5 hours after an oral intake of either form of the vitamin and this increase was independent of the form given. A constant ratio between dehydroascorbic acid and ascorbic acid often was maintained for several hours after an oral intake of either form of the vitamin; however, such was not always true. The results for three subjects with different fasting concentrations of dehydroascorbic acid were as follows. The dehydroascorbic acid (0.65 mg./100 ml.) for subject A was extremely high and a considerable fraction (41 per cent) of the total. After an intake of 300 mg. dehydroascorbic acid, this high percentage dropped only slightly (31 to 33 per cent) during a period of five hours. In sharp contrast, the initial value of 7 per cent for subject C was maintained even though an intake of 500 mg. dehydroascorbic acid had more than doubled the fasting total ascorbic acid. In subject B, the initial concentration of dehydroascorbic acid (16 per cent of the total) increased to 31 per cent after an intake of 150 mg. ascorbic acid.

C. P. Stewart, D. B. Horn and J. S. Robson (*Biochem. J.* **53,** *254* (*1953*)) have indicated that adrenocortical steroids influence the ratio between dehydroascorbic acid and ascorbic acid. They reported that both ascorbic and ascorbic plus dehydroascorbic acid in plasma were elevated in normal human subjects by the administration of ACTH. The larger increases in ascorbic acid were accompanied by a diminution of dehydroascorbic acid. Cortisone caused a complete disappearance of dehydroascorbic acid without changing the total (concentration) of ascorbic acid.

It can be concluded that the total concentration of ascorbic acid in the serum (ascorbic acid plus dehydroascorbic acid) is consistently greater than that of the reduced form alone. Following ingestion of either ascorbic acid or dehydroascorbic acid there is a rapid rise of the two acids in the serum, the increase being independent of the form in which the vitamin is given. The original fasting ratio existing between the two forms often remains remarkably constant after ingestion of either form of the vitamin and is possibly a function of, as yet obscure, hormonal controls.

PROTEIN METABOLISM DURING CORTICOSTEROID THERAPY

Most statements concerning the therapeutic use of hydrocortisone include the comment that the steroid has either a protein catabolic or anti-anabolic effect. E. C. Reifenstein (*Metabolism* **7,** *78* (*1958*)) in a symposium on adrenal corticosteroids stated

that all patients receiving maintenance doses of these steroids should be given at the same time a protein anabolic hormone (*e.g.* one of the testosterone derivatives to prevent the accelerated rate of breakdown of body protein).

However, recently H. F. West (*Lancet* **II**, *877 (1958)*) has noted that it is not uncommon for patients to show increased strength and weight during adrenal corticosteroid therapy. In fact, this is usually the case for the debilitated patient with rheumatoid arthritis and for those with Addison's disease. During the course of other work this investigator had reviewed a large number of 24 hour urine creatinine excretion rates in patients who were receiving prolonged corticosteroid therapy.

The creatinine determinations were being done to check the completeness of the urinary collection. Urinary excretion of creatinine is believed to be proportional to the amount of creatinine in the body. Since approximately 98 per cent is in skeletal muscle, one might conclude that the excretion of creatinine reflects the magnitude of the muscle mass. This is substantiated in part by the fact that in no chronic disease state, with the possible exception of acromegaly, have changes in the excretion of creatinine been shown to be at variance with changes in muscle mass.

The small day-to-day variation which was found to occur in the 24 hour creatinine level, even under the most exact collection procedures, made it necessary to obtain a large number of specimens to obtain reliable mean values for a given individual. It was possible to obtain a sufficient number of specimens of the required degree of accuracy in 19 patients with rheumatoid arthritis (seven men and twelve women). The mean age at the start of steroid therapy for this group was 38 years (16 to 68) and the mean duration of their disease was five and one-half years (four months to 17 years). The majority of the patients received corticotropin at a dose level which would increase the output of urinary 17-ketogenic steroids or 17-hydroxy corticosteroids 100 to 200 per cent in excess of control values. While most of the patients received corticotropin, some were given cortisone acetate or prednisolone. Cortisone acetate was administered at a level of 50 mg. per day in divided doses, and where used, 12.5 mg. or more of prednisolone were given per day.

During this study findings were considered only for those patients in whom a sufficient number of creatinine analyses were obtained to justify statistical analyses. Significant changes from the control value creatinine levels were noted in 29 of the 68 periods of adrenal steroid or corticotropin administration. The creatinine level increased in 21 and decreased in eight of the 29 periods. Thus, there were increases in muscle mass during 21 periods and decreases in eight.

Another method in presenting these data was to compare only the changes between the control and the final period since, if any pronounced cumulative effect of the hormone was present, the difference would be maximal over the long period. In 11 of the 19 patients, no significant difference was noted in their urinary creatinine levels during the control period as compared with the final period of study. Six of the 11 patients had been on steroid therapy from two to five years. Increases in creatinine excretions were noted in six patients who had been on therapy from one-half to five years and decreases were noted in only two patients who received these substances for three to four years.

One of the two latter patients showed significant increases in creatinine levels in urine during at least one period, and this can be interpreted as an increase in muscle mass during this time. Thirteen of the nineteen patients treated had increases in muscle mass, as indicated by increases in the urinary creatinine levels at some time during the course of therapy, and only one had a decrease.

In some instances where there was a decrease in muscle mass, these changes could be more readily explained by factors other than the protein degradation effect of the steroids (conditions which would influence the patient's food consumption, *i.e.* emotional upsets, increased crippling of the hands which would make eating difficult) or because the patients had become so crippled from their disease that more muscular deterioration occurred as a result of disuse atrophy.

These investigators believe that the long term use of corticosteroid, in doses employed for the treatment of patients with rheumatoid arthritis, is likely to be accompanied by an increase in muscle mass providing the patient is eating well and is not sufficiently crippled to develop disuse atrophy or wasting. While short term balance studies have indicated that protein breakdown occurs during corticosteroid therapy at higher dosage levels and that these effects can be counteracted by testosterone or some of its derivatives, it does not follow that such events may necessarily continue during long periods on lower dose levels.

It has been stated that the protein catabolic effects of corticosteroid therapy are normally balanced by increased appetite and food intake of the patient. Thus, West does not believe that one need routinely employ protein anabolic hormones when corticosteroids are used in therapy at the dose levels employed in his present study to prevent decreases in muscle mass of the body.

BUFFERED NICOTINIC ACID AND SERUM CHOLESTEROL

Experiments have been reported describing the use of high dosages of nicotinic acid (44 mg. per kg. per day) to produce decreases of serum cholesterol in man and rabbits (R. Altschul and A. Hoffer, *Arch. Biochem.* **73,** *420* (*1958*)).

The possibility that such a high dosage might be toxic to the liver was examined in patients receiving the drug over long periods. Nothing abnormal was seen either in liver function tests or other routine examinations. K. Unna (*J. Pharmacol. Exp. Therap.* **65,** *95* (*1939*)) in commenting on the toxicity of nicotinic acid reported by C. A. Elvehjem, *et al.*, (*J. Biol. Chem.* **123,** *137* (*1938*)) proposed that the toxicity was due mainly to the high acidity of the compound. In spite of the suggestion that the sodium salt of nicotinic acid might be better tolerated by experimental animals, reports continued to appear describing the specific toxic effects of nicotinic acid.

P. Handler and W. J. Dann (*J. Biol. Chem.* **146,** *357* (*1942*)), for example, fed rats a diet containing 2 per cent nicotinic acid and found that the animals gained weight and showed an increase in the fatty acid content of their livers. On the other hand, nicotinamide did not promote fatty liver production but resulted in growth arrest. A. Aschkenasy and J. Mignot (*C. R. Soc. Biol.* (*Paris*) **140,** *261* (*1946*)) found that nicotinamide produced necrotic foci in the livers and increased fat but caused no arrest of growth. R. G. Janes (*Am. J. Physiol.* **174,** *46* (*1953*)) found no change in liver lipids resulting from injection of nicotinic acid into rats.

To study further this problem of acidity, R. Altschul and A. Hoffer (*Brit. Med. J.* **2,** *713* (*1958*)) have performed experiments in which they have fed 1 g. of buffered nicotinic acid to healthy young adults three times a day for two weeks. Preliminary experiments in which they fed rabbits and rats high doses of nicotinic acid resulted in no morphological or biochemical changes. The fact that some human cases treated with nicotinic acid had to have the drug discontinued because of gastrointestinal disturbances (R. W. P. Achor, *et al.*, *Circulation* **16,** *499* (*1957*)) prompted R. Altschul and

A. Hoffer to test the acceptability of buffered nicotinic acid and to determine whether it was as effective in reducing the level of serum cholesterol.

Twelve subjects were treated with the buffered nicotinic acid for two weeks. During this interval the mean cholesterol level dropped from 201 mg. to 162 mg. per 100 ml. The difference was highly significant. Six of the same subjects had ingested 3 g. of unbuffered nicotinic acid in an earlier experiment. The mean cholesterol levels had decreased from 207 to 163 mg. per cent.

These changes in cholesterol with the two forms of the drug are nearly identical and indicate that either nicotinic acid or its neutral salts can be used to reduce cholesterol levels.

If vascular dilatation occurs as a side effect, ingesting the 1 g. dose in small amounts over a period of an hour may reduce these symptoms. If sodium restriction is desirable potassium bicarbonate may be substituted.

Summary: The buffered salts of nicotinic acid appear to be capable of reducing serum cholesterol levels as well as nicotinic acid and the effects of undesirable acidity are avoided.

Further experimental and clinical study is indicated to resolve the question of hepatic toxicity of this drug.

EXCRETION OF HYDROXYPROLINE IN MARFAN'S SYNDROME

The distribution of the amino acid hydroxyproline is unique since almost the entire content of the body is present in collagen. In fact, the determination of this amino acid has been used to estimate the amount of collagen in various tissues. The possibility occurs that if there were large deviations in the metabolism of collagen, perhaps altered amounts of this amino acid would appear in the urine. M. Ziff, A. Kibrick, E. Dresner and H. J. Grivetz (*J. Clin. Invest.* **35,** *579 (1956)*) could not find any abnormal excretion of hydroxyproline in the urine of a group of patients with one or another of the collagen diseases.

Recently, A. Sjoerdsma, J. D. Davidson, S. Udenfriend and Z. Mitoma (*Lancet* **II,** *994 (1958)*) have reported, in preliminary form, that patients with Marfan's syndrome have been found to excrete increased amounts of hydroxyproline in the urine. Individuals with this syndrome are believed to have a defect in the connective tissue, possibly in the elastic tissue. In addition to defects in the skeleton and eye, the aorta may also be involved. There may be degeneration of the media of the aorta with the formation of aneurysms.

For this study, the patients were maintained on an unrestricted diet when the 24 hour urine was obtained. The total urinary hydroxyproline content was determined. The twelve normal patients studied excreted 14 to 38 mg. of hydroxyproline per 24 hours. A similar amount of hydroxyproline was found in the urine of 38 patients who had a group of miscellaneous disorders including rheumatoid arthritis, scleroderma, atherosclerotic and hypertensive cardiovascular disease, asthma, regional ileitis, ulcerative colitis and Hodgkin's disease.

In eight individuals with Marfan's syndrome (seven men and one woman) the daily excretion of hydroxyproline ranged from 106 to 30 mg. per day with six of the eight values being over 56 mg. per day. Two additional cases, one male and one female, in which there was reasonable doubt about the clinical diagnosis of Marfan's syndrome, had 24 hour hydroxyproline excretions of 22 and 18 mg.

Since these subjects were on unrestricted diets at the time the urine collections were being made, the question arises as to whether or not the ingestion of gelatin, which would occur in many hospital diets, would have any effect upon the excretion of hydroxyproline in the urine. Also, in the preliminary report, no information was given as to the

methods employed for the determination of urinary hydroxyproline; this is of importance since there is some discrepancy at present in regard to the various techniques used.

Thus, it appears that at least some patients with this syndrome excrete abnormally large amounts of hydroxyproline in the urine. Whether the increased excretion of hydroxyproline is the result of a general metabolic disturbance or a phenomenon related directly to the kidney, cannot be determined at present. Also, the question arises as to the excretion of hydroxyproline in other members of the patient's family who do not have the syndrome. When more information is obtained in regard to these latter two points, it may be possible to establish the mechanism responsible for the increased urinary excretion of hydroxyproline.

5-HYDROXYTRYPTOPHAN DECARBOXYLASE AND PHENYLKETONURIA

The biochemical changes in patients with phenylketonuria (phenylpyruvica oligophrenia) have been reviewed earlier (*Nutrition Reviews* **15,** *150* (*1957*)). It has been shown that the error appears to be failure of hydroxylation of phenylalanine to tyrosine. Later, it was reported that individuals with phenylketonuria also excreted indoleacetic acid into their urine, leading M. D. Armstrong and K. S. Robinson (*Arch. Biochem.* **52,** *287* (*1954*)) to conclude that these patients also had a defect in the metabolism of tryptophan.

Then it was suggested that patients with phenylketonuria have a defect in the 5-hydroxyindole pathway of the metabolism of tryptophan (C. M. D. Pare, M. Sandler and R. S. Stacey, *Lancet* **I,** *551* (*1957*)). These investigators found significantly lower levels of serotonin in the blood and also low levels of its metabolite, 5-hydroxyindoleacetic acid, in the urine. An enzyme which converts 5-hydroxytryptophan to serotonin, 5-hydroxytryptophan decarboxylase, was found by A. N. Davidson and M. Sandler (*Nature* **181,** *186* (*1957*)) to be inhibited *in vitro* by certain of the metabolites of phenylalanine.

Recently, Pare, Sandler and Stacey (*Lancet* **II,** *1299* (*1958*)) have postulated that if the inhibition of 5-hydroxytryptophan decarboxylase were to occur *in vivo*, it might explain the findings of the defect in tryptophan metabolism in patients with phenylketonuria. Also, reducing the metabolites of phenylalanine in these patients by maintaining them on a low phenylalanine diet might induce increased production of serotonin and its metabolite, 5-hydroxyindoleacetic acid.

To investigate this, seven subjects with phenylketonuria were studied before, and during a phenylalanine low diet. Serum serotonin, urine 5-hydroxyindoleacetic acid, creatinine and the indole excretion pattern were studied. In seven patients who were maintained on the low phenylalanine diet for four weeks, it was found that the serum serotonin values were significantly higher than before going on the special diet. On the other hand, the urinary excretion of 5-hydroxyindoleacetic acid was no more statistically significant at the end of four weeks on the low phenylalanine diet than before. The average rise in serum serotonin levels increased progressively for each of the weeks studied and the results could be plotted as a straight line against time.

In addition, a 5-hydroxytryptophan tolerance test was carried out in four phenylketonuric children and four mentally defective children without phenylketonuria, matched for age, sex, and nutritional state. All the individuals were living in the same hospital and receiving a regular diet. In carrying out this test, each individual received 25 mg. of DL 5-hydroxytryptophan intravenously and their urine was collected

for time periods before and after the test. Since it was not certain that complete urine samples were being obtained, the investigators expressed their values in mg. of 5-hydroxyindoleacetic acid per gram of creatinine.

The phenylketonuric children were found to have consistently lower excretion peaks for serotonin and 5-hydroxyindoleacetic acid after the administration of the 5-hydroxytryptophan than did the matched control children. By the use of both the low phenylalanine diet, which decreased the amount of inhibitors for 5-hydroxytryptophan decarboxylase (thus allowing increased amounts of serotonin to be formed), and the 5-hydroxytryptophan tolerance test, which showed that the patients with phenylketonuria could not decarboxylate the amino acid as well as the control patients, these investigators concluded that a diminished decarboxylation of 5-hydroxytryptophan occurs in phenylketonuria.

Since mental deficiency is a common factor present in phenylketonuria, the question arises as to the relationship of the amino acid defect to the mental ability of the patient. Pare and associates have suggested that the mental deficiency in phenylketonuria is associated with an impaired serotonin synthesis rather than any specific action of toxic metabolites of phenylalanine.

The low phenylalanine diet appears to be most effective when applied to the youngest children. The possibility arises that long exposure to excessive phenylalanine eventually results in irreversible changes. While the interrelationship of amino acids has been stressed (particularly in nutritional works where weight gain and changes in the liver are noted) perhaps a broader aspect of the problem of amino acid imbalance is present in phenylketonuria. Here, perhaps, an imbalance between the metabolites of phenylalanine and tryptophan may lead to changes culminating in mental deficiency in these individuals.

DINITROPHENOL AND DIABETES

The fact that both salicylate and 2:4-dinitrophenol (DNP) inhibit oxidative phosphorylation prompted J. Reid (*Brit. Med. J.* **2,** *724* (*1958*)) to compare the action of these two drugs in the treatment of diabetic patients. The use of aspirin to control some of the metabolic abnormalities of diabetes has been the subject of a recent review (*Nutrition Reviews* **16,** *167* (*1958*)).

DNP, a powerful metabolic stimulant, was misused as a weight reducing agent in the 1930's and the high incidence of toxic effects led to its withdrawal as a therapeutic agent.

In order to study the effect of DNP on blood sugar, glycosuria and glucose tolerance, a brief, carefully controlled trial was carried out. The subjects, six elderly ladies with mild to moderately severe diabetes, were fed a constant, low-carbohydrate diet for ten days before starting DNP in order to determine the effect of diet alone. This diet, containing a daily allowance of only 150 g. of carbohydrate, was continued throughout the experiment. From 100 to 300 mg. of DNP were given daily for 14 days, the dosage being determined by analysis of the plasma level and by the measurement of oxygen consumption to determine the basal metabolic rate.

The average value for plasma DNP concentration ranged between 34 and 48 mg. per liter. As the plasma level rose the basal metabolic rate also increased. No toxic symptoms were noted and the degree of stimulation of metabolism was similar to that seen with aspirin.

Fasting blood sugars determined before administration of DNP were stable over a period of several days. The average blood

glucose for the group was 246 mg. per 100 ml. on the day before DNP was started, and 186 mg. per 100 ml. after 14 days of DNP. The employment of the t-test gave a p value between 0.05 and 0.02, indicating that the decrease in mean fasting blood sugar was significant.

Urine glucose was measured before, during, and after DNP and the trend was similar to that of the blood glucose levels. The average urine glucose was 20 g. excreted during the 24 hours prior to DNP administration and 11 g. at the end of the DNP treatment. The p value was between 0.02 and 0.01, demonstrating that the decrease in glycosuria was significant.

To determine glucose tolerance before and after DNP 50 g. of glucose were fed. The improvement was limited to two patients and was slight in one while moderate in the other.

Body weights before DNP were steady, but at the end of 14 days three patients lost 1.3 to 2.1 kg. During the five-day period after DNP there was no significant change in weight. This was in contrast to the case of salicylate, where weight loss occurred during a five day period after stopping treatment.

The results suggested a similarity between the therapeutic effects of aspirin and DNP, but salicylate appeared to be more potent. In order to compare the action of the two drugs directly, four of the patients given DNP for 14 days were treated with aspirin after allowing a few days for disappearance of DNP from the plasma. The actions of DNP and aspirin were compared on fasting blood sugar and glucose tolerance. The doses of aspirin reported previously ranged from 1.0 to 1.6 g. every four hours except in the middle of the night, whereas the dose of DNP was 100 to 300 mg. daily. The average value of blood sugar for these four patients of 272 mg. per 100 ml. before DNP fell to 211 mg. by the end of 14 days. The average blood sugar level before aspirin of 209 mg. per 100 ml. fell to 99 mg. during 14 days. Thus, in spite of a lower pretreatment blood glucose level, the decrease following aspirin therapy was greater.

The glucose tolerance test using 50 g. of glucose was performed in three patients before and after DNP, and then consecutively before and after aspirin. One patient showed slight improvement after DNP. After aspirin, however, all three showed pronounced improvement.

A study of basal metabolic rate compared with fasting blood sugar in individual patients showed that increased oxygen consumption resulting from DNP administration did not correlate with a reduction in the fasting blood sugar below a certain limit. A similar increase in the basal metabolic rate following aspirin therapy was associated with a decrease of the fasting blood sugar to normal.

In view of the possibility that the effect of aspirin could be related to the fact that its use followed DNP therapy, it was decided to compare the effect of each drug in separate but comparable groups of patients. The DNP effect was evaluated in the group of patients discussed in the early part of this report. The second group which received aspirin had been the subject of an earlier report (J. Reid, A. I. Macdougall and M. M. Andrews, *Brit. Med. J.* **2**, *1071 (1957)*).

Both groups of patients were similar clinically in that they had mild to moderately severe diabetes. The average, fasting blood sugar was 245 mg. per 100 ml. before giving DNP and 190 mg. before giving aspirin. The difference was not significant. The average daily carbohydrate intake for the DNP group was 150 g. compared to 130 g. for the aspirin group. This difference in intake might account for the difference in blood sugar. During the treatment period with aspirin the blood sugar fell from 190 to 92 mg. per 100 ml., whereas the DNP treatment caused the blood sugar to fall from 245 to 186 mg. per 100 ml. during 14 days.

The effect of DNP in reducing blood sugar

is contrary to the findings reported by C. M. MacBryde and B. L. Taussig (*J. Am. Med. Assoc.* **105,** *13 (1935)*) who observed a slight rise in fasting blood sugar and decreased glucose tolerance in three cases given DNP. The fact that two patients were pre-diabetic may account for the difference. Perhaps the milder the diabetes, the less likely is the blood sugar to fall. J. B. Cochran, R. D. Watson and J. Reid (*Brit. Med. J.* **2,** *1411 (1950)*) have observed that non-diabetic rheumatic patients may show a rise in fasting blood sugar and decreased glucose tolerance while receiving salicylate. This suggests that a patient's reaction to salicylate or DNP administration may have diagnostic value in detecting the diabetic state before glycosuria is evident.

Both aspirin and DNP increase oxygen consumption and both lower fasting blood sugar, but the effect of aspirin is greater. The results indicate that the use of DNP clinically would probably not be practical. It would seem unwarranted to postulate the inhibition of oxidative phosphorylation as the fundamental action of each of these compounds without further attempting to characterize the quantitative differences in their action.

Summary: Clinical comparison of the therapeutic effects of salicylate and 2:4-dinitrophenol (DNP) on moderately severe diabetes demonstrated that both reduced fasting blood sugar and glycosuria. With doses within tolerance limits, aspirin was more effective and DNP would probably have no practical value. The fact that both caused inhibition of oxidative phosphorylation was not sufficient to explain the quantitative differences.

VITAMIN D ACTIVITY IN ROUGHAGES

In areas of the world where there is little ultra-violet irradiation for much of the year, grazing animals rely, to an important extent, on the vitamin D activity of the foods that they eat. At the same time it is inconvenient and expensive to give these animals concentrated supplements containing vitamin D concentrates. There is, therefore, a practical as well as a fundamental interest in studies of the occurrence of this vitamin in plant tissues, even at very low concentrations, and the further synthesis in naturally irradiated dead tissue.

In general terms it is well established that the vitamin D activity of fresh herbage is low, and the activity will be increased when the herbage is conserved as hay. The literature has been reviewed by J. Weits (*Mededelingen de Landbouwhogelschool te Wageningen* **56,** *1 (1956)*), who concludes that the factors influencing the relative vitamin D activity of different roughages are still not fully understood.

In his own studies Weits (*loc. cit.*) has confirmed that the apparent activity of samples may differ from the true vitamin D content because of the presence of interfering materials. For example, the addition of hay to a rachitogenic diet for rats can result in the introduction of sufficient additional calcium and phosphorus to change the response to vitamin D to a significant extent. However the interfering factors seem to be confined to the lipid and ash fractions since the activity of "hay fat" + "hay ash" in a "curative" assay with rachitic rats receiving a basal diet of the Steenbock type was equal to that of the equivalent amount of whole hay. The activity of the lipid fraction alone was only 50 to 70 per cent that of the whole hay.

Further trials indicated that simple extraction of hay samples with ether failed to release their full vitamin D content. Higher values were obtained with a preliminary digestion of the samples in 10 per cent potassium hydroxide solution and then extraction with petroleum ether. It did not

prove possible to extract more active material from the residues from this procedure. For six samples of hay the overall mean activity, when assays were carried out with simple ether-extracts was 730 I.U. per kg., and when carried out with petroleum ether extracts after alkaline digestion it was 950 I.U. per kg. of hay.

The true vitamin D content of the extracts was, however, greater than indicated by their direct activity in rat assays. The presence of inhibitors was first suggested by the "standard" and "hay extract" regression lines having a different slope in the assays. This was confirmed directly by further purification of the extracts by chromatography on alumina columns. The interfering agents could be removed with petroleum ether, and then the vitamin D could be eluted separately with petroleum ether containing 0.5 per cent of added ethanol.

Weits (*loc. cit.*) considers that there is sufficient evidence to indict carotene as the main anti-vitamin D factor in the herbage. In controlled tests, daily doses of 40 to 60 μg. of carotene reduced the response to vitamin D by approximately 30 per cent under the conditions of a "curative" assay. No such effect was found when newly weaned rats were given the rachitogenic diet with "preventive" supplements.

Applying the "curative" assay procedure to purified extracts from various Dutch roughages, Weits found eight samples of dehydrated grass to have values ranging from 70 to 720 I.U. vitamin D per kg. of dry matter with a mean of 340 I.U. per kg. (though a ninth sample gave an unexpected value of 1,500 I.U. per kg.). On a dry matter basis again, samples of ensiled grass gave a similar range of values, as would be expected from a method of preservation in which there is no access to radiation.

In contrast, ten samples of grass hay cured in the field without special equipment had a mean value of 990 I.U. per kg. of dry matter. However there was a very wide range of individual values from 420 to 1,810 I.U. per kg. The fiducial limits (P = 0.05) were approximately 70 to 140 per cent of each calculated value. Samples of barn-cured and frame-dried hay had a lower mean value of 800 I.U. per kg., but there was a considerable overlap for individual values between these and the field-cured hays.

G. C. Wallis, G. H. Kennedy and R. H. Fishman (*J. Animal Sci.* **17,** *410 (1958)*) have recently reported the results of large collaborative assays of North American roughages. In these trials, rat assays were deliberately carried out with the whole samples in order to obtain their "net vitamin D activity for the animal (true potency minus antivitamin D effects)". Forty samples of sun-cured hays gave values ranging from 150 to 3,200 I.U. per kg. The wide range was not to be explained by species differences, since the alfalfa samples within the series gave almost the full width of scatter. Nor could the results be related to the climatic conditions during curing. Government grading of the samples by inspection also showed little relation to their vitamin D activity. Again there was no certainty that individual samples of mow-cured hay would differ in activity from apparently comparable sun-cured materials.

More values have also been published for the vitamin D activity of British pastures and hays (K. M. Henry, *et al., Brit. J. Nutrition* **12,** *462 (1958)*). These workers used "preventive" rat assays with a rachitogenic diet supplemented with extracts of non-saponifiable matter from the test materials. Their first experiments confirmed the finding of Weits (*loc. cit.*) that a preliminary alkaline digestion increased the activity of the extract to be obtained with petroleum ether. No further separation of vitamin D was attempted.

In the first series of trials, fresh monthly samples were cut from April to November for three years from a Scottish hill pasture. Values ranged from 50 to 1,000 I.U. per kg. of air-dried material, and there was no consistent pattern of seasonal effects.

In the second series, samples were taken in three successive years from the same lowland field, of the original sward at the time of cutting, and of both ground-cured and tripod hay prepared from it. The sward gave values ranging from 40 to 320 I.U. of vitamin D per kg. of air-dried material; the ground-cured hays showed 340 to 1630 I.U. of activity and the tripod hays 190 to 590 I.U.

In all three of these studies carried out with different assay techniques, and with samples taken from different agricultural systems, the general conclusions are similar, and in agreement with the earlier findings of J. A. Newlander (*J. Dairy Sci.* **31,** *455 (1948)*) and of others, that it is unsafe to conclude that an individual sample of hay, regardless of its known period or intensity of irradiation, will be a particularly rich source of vitamin D activity. The reasons for the variations observed have still to be worked out. Nor is it certain that the occasional relatively high values for intact plants are due entirely to adhering dead tissues in which the vitamin has been synthesised as a result of irradiation.

GROWTH HORMONE AND SKELETAL CHANGES IN VITAMIN A DEFICIENCY

Nutritional deficiency may result in symptoms that are either directly or indirectly caused by a dietary lack. For example L. A. Bavetta, S. Bernick and B. H. Ershoff (*Endocrinology* **58,** *701 (1956)*) in studying tryptophan-deficient rats found that growth hormone injections, although unable to influence the retardation of growth, corrected the impairment of chondrogenesis and osteogenesis of the tibia and alveolar bone. The effect of growth hormone on cartilage metabolism has been the subject of recent reviews (*Nutrition Reviews* **14,** *26 (1956)*; **15,** *349 (1957)*). These authors have also studied the effect of growth hormone on abnormalities in the bone and periodontium of rats depleted of vitamin A (L. A. Bavetta, S. Bernick and B. H. Ershoff, *Arch. Path.* **66,** *610 (1958)*).

In order to produce vitamin A deficient young animals the females were kept on a special diet for two months before breeding and continuing for seven days after birth of the litter (H. C. Sherman and H. L. Campbell, *J. Biol. Chem.* **60,** *5 (1924)*). After this a vitamin A-low diet was given both mothers and young from seven days to 21 days at which time the animals were weaned and 40 females were chosen for the experiment.

Thirty rats were fed a vitamin A-deficient diet containing sucrose, casein, corn oil, salts and vitamins. Ten rats were fed the same diet but including vitamin A. The feeding was continued for five days after the animals stopped gaining weight or started losing, the average time required being about 22 days after weaning. These rats were then divided into two groups of 12 rats each, half receiving ten daily intraperitoneal injections of saline and half given similar injections each containing 100 micrograms of growth hormone. Nine of the saline-treated and seven of the hormone-treated animals survived till the time of autopsy, 24 hours after the last injection.

Growth hormone produced no acceleration in weight gain in the vitamin A-deficient animals, in agreement with earlier findings.

The vitamin A-deficient animals receiving saline showed a decrease in the cartilaginous epiphyseal plate of the tibia due to a decrease in the number and size of cells.

Trabeculae were lost in both epiphysis and diaphysis. In contrast, the growth hormone-treated animals showed increased chondrogenic activity in all layers of the epiphyseal plate. Measurements of the epiphyseal cartilage of rats given saline gave an average width of 190 micra compared with

290 micra in deficient rats given growth hormone. The cartilage of animals with adequate vitamin A intake measured 390 micra.

The periodontium and mandibular condyle of saline treated rats also showed a decrease in the numbers of cartilage cells due to vitamin A deficiency. Growth hormone, however, reversed the changes in a manner similar to the stimulation seen in the tibia but animals getting vitamin A from weaning showed wider cartilage and denser bone.

Osteoporosis of the spongiosa of the periodontium was typical of all rats injected with saline. Growth hormone promoted bone deposition to such a degree that marrow spaces were nearly obliterated. Vitamin A-sufficient animals had well developed trabeculae and marrow, well filled with myeloid tissue.

The incisal dentin found during the experimental period in vitamin A-deficient rats showed an abnormal number of interglobular spaces and the zone of uncalcified predentin appeared wider than normal. In growth hormone-injected rats there was a decrease in the size and number of interglobular spaces and a normal width of predentin. The animals getting adequate vitamin A had a homogeneous dentin.

Summary: Vitamin A-depleted rats given growth hormone showed no increment in body weight but restored chondrogenesis in the epiphyseal region of the tibia, bone formation in alveolar bone and formation of normal appearing incisal dentin. The authors suggest that the impairment in chondrogenesis and osteogenesis was at least partly due to either impaired production or decreased secretion of growth hormone during vitamin A deficiency. The well known sensitivity of the skeleton to growth hormone may be a more important factor in this differential effect.

LIPID REQUIREMENTS OF A MICROORGANISM

In many instances, studies with microorganisms have revealed essential nutrients for higher animals. Especially in the case of the B-vitamins, their mechanism of action as co-factors in vital enzymatic reactions is often the same for most living organisms. No such claim can be made for the fat-soluble nutrients, however. These appear to be associated with higher organization, and where lipids are found to be necessary for microorganisms, their use often appears to have little relationship to that in mammals (e.g. the requirement of oleic acid by certain lactobacilli, *Nutrition Reviews* **16,** *116 (1958)*).

A case in point is found in the lipid requirement of pleuropneumonia-like organisms (PPLO). P. F. Smith, J. G. Lecce and R. J. Lynn (*J. Bacteriol.* **68,** *627 (1954)*) found that the factor in mammalian blood sera necessary for growth of this organism is a lipoprotein. All three of the major components of this lipoprotein (protein, cholesterol and lecithin) were found to be necessary, although the only requirement for the protein appeared to be its ability to bind cholesterol.

In order to investigate the lipid requirement in greater detail, Smith and Lynn (*J. Bacteriol.* **76,** *264 (1958)*) tested the activity of a great variety of lipids in promotion of growth of PPLO. The organisms were grown on an agar medium supplemented with PPLO serum fractions. Tests were conducted in a liquid test medium either unextracted or ether-extracted to which were added a methanol-extracted protein supplement and either cholesterol or lecithin. The growth in these experimental suspensions was compared with that in unextracted broth supplemented with unextracted protein.

Of a great variety of steroids and related compounds tested, only β-sitosterol and dihydrocholesterol were as effective in

growth promotion as cholesterol. A few compounds, such as ergosterol and certain cholesterol esters, had weak activity. Others, such as the known precursors of cholesterol (mevalonic acid and squalene), 7-dehydrocholesterol and many unrelated compounds had no activity. Still others, such as cholestane and desoxycholic acid, were actually inhibitory. The authors concluded that for growth-promoting activity, the 3-hydroxy group, the cyclopentanophenanthrene ring system and the side chain were absolute requirements and that even minor alterations resulted in loss of activity.

The phospholipid requirement could be met by lecithin and, to a lesser extent, by sodium cholate. Since about the only common property of these substances is their surface activity, it was thought possible that the effect of this component was in solubilization of the cholesterol.

Even with supplementary cholesterol and lecithin, the extracted medium did not have the full growth promoting activity of the unextracted medium. It was found that certain short chain fatty acids such as acetate, butyrate or, especially, a combination of the two, could bring the activity up to that of the control.

The function of these lipids in promoting growth in these organisms is still not clear. It is improbable that cholesterol serves as a precursor for other steroids as in the mammal, and it is certainly not involved in lipid transport. It seems probable that the intact lipoprotein may be the actual requirement, but even in this case, clarification must await the results of further experiments.

ALTERNATE HIGH- AND LOW-PROTEIN FEEDING

To utilize the protein in the diet, an animal must receive all the essential amino acids. An accumulation of experimental work shows that an animal, receiving all the essential amino acids in the course of each twenty-four hour period but not all at the same feeding, will not grow well (E. Geiger, *J. Nutrition* **34,** *97 (1947)*).

This work was extended to mixtures of proteins, each of which was deficient in one or more essential amino acids, but which, when fed together, provided all the essential amino acids. Geiger, at the University of Southern California (*Ibid.* **36,** *813 (1948)*), found that when rats were fed a ration containing two such incomplete proteins the animals grew at a normal rate. However, if the individual proteins were fed at different times of the day, the animals did not grow.

Geiger used a purified basal diet containing adequate amounts of salts and minerals. One group of rats received the basal diet containing 9 per cent blood protein during the hours of 8 a.m. to 6 p.m., while from 8 p.m. to 6 a.m. these animals received the basal diet containing 9 per cent wheat gluten. Another group received the basal diet containing both blood protein and wheat gluten, each at a level of 4.5 per cent; the latter was available to the rats at all hours. Paired feeding was used to equalize the food intake.

Over the 16-day experimental period, the four weanling male rats receiving one protein during the day and the other during the night gained no weight, whereas the animals fed the diet containing a mixture of the two proteins gained an average of 22 g. (E. Geiger, *loc. cit.*)

Similar experiments were carried out with mixtures of yeast plus blood protein and yeast plus wheat gluten. In all cases, the animals fed the diet containing the mixture of proteins gained approximately 20 g. during the 16-day experimental period, whereas the animals fed the two proteins at separate times of the day either lost weight or showed only a very slight gain. These differences in weight gain occurred in spite of the fact that

the food intake was the same for each of the two groups in both experiments.

The work of Geiger indicates that two proteins, supplementing each other's amino acid deficiency, are effective in promoting growth only when fed simultaneously.

A. van Dam-Bakker, A. P. DeGroot and R. Luyken of Utrecht, The Netherlands (*Brit. J. Nutrition* **12,** *259 (1958)*), were interested in studying a situation which was still more extreme than the one just described. They attempted to simulate the condition existing in some tropical areas, where a high protein diet is consumed for a brief period after a day of hunting or fishing. The latter is frequently followed by a protracted period when only carbohydrates (in the form of the starch, sago) are consumed.

These workers used two groups of weanling rats, with 24 animals in each group (12 males and 12 females). One group was given, alternately, a high protein diet and a low protein diet. The other group was given a mixture of these two. The high protein diet consisted of equal parts of commercial fish meal and potato protein (the by-product of the potato-starch industry). The low protein diet consisted of wheat starch, soy bean oil, and adequate minerals and vitamins. The control group received a mixture of these two diets in the ratio of 3:7 and the protein content of this diet was 22.4 per cent. The experimental group was fed the low protein diet during the night. The amount consumed was measured. During the day these animals were given the high protein diet in an amount which equaled 3/7 of the weight of the low protein diet consumed. The control animals received the mixture of low and high protein diets in an amount equal to that consumed by the experimental group.

The male rats fed on the mixed diet gained 403 g. during the 284 experimental days, whereas those fed the high and low protein diets at different intervals of the day gained 332 g. At the end of 161 days, the females on the control diet gained 215 g., while those fed the high and low protein diets at different intervals gained 176 g.

The offspring of the above rats were maintained on the same diets as their parents. Both of these groups of animals showed a much slower rate of weight gain than their parents. The males on the mixed diet gained 185 g. by the eighty-fourth day versus 259 g. for their parents. The males on the alternate feeding regimen gained 148 g. versus 201 g. for their parents.

Three consecutive matings of the parent generation and two matings of the first generation indicated that there was no difference in the number of litters for the rats fed the mixed diet compared to those fed the two diets at different times of the day. For the five matings, 95 per cent of the females in the experimental group produced litters, while 97 per cent did so in the control group. The total number of pups per litter at birth, the weight of the pups, and their mortality did not show any consistent differences for the two groups. The young of the mothers fed the two diets at different times of the day weighed considerably less when weaned than did those of the control animals (17 versus 26 g.). That the poor gain of the pups during the weaning period was due to lactation difficulties on the part of the mother was shown when the pups from rats fed alternately the high- and low-protein diet were given to normal wet nurses. Under these circumstances, the young gained the same as those of mothers on the normal diet.

The poor nutritional condition of the weanling animals born to the rats fed alternately the high and low protein diet was apparent even when they were put on a stock ration immediately after weaning. Under these circumstances, the male offspring of the alternately fed rats gained 269 g. at the end of 154 days, whereas the offspring of the control rats gained 307 g. The corresponding values for the females were 164 g. versus 185 g. The above observation strongly suggests that nutritional impairment occurring during the pre-weaning period may carry through a large share of the animal's life, even though it is fed an adequate diet after weaning.

The hemoglobin level in the parent rats fed the two diets alternately was 14.4 g. per 100 ml. of blood, whereas that of the control rats was 13.7. Although the hemoglobin levels were the same in both groups, the alternately fed animals were able to recover from severe anemia produced by phenylhydrazine at a slower rate than the control animals. In the latter work, the control animals showed an increase in hemoglobin from 4.5 to 14.2 g. in 11 days, whereas the alternately fed animals required an additional week in order to restore their hemoglobin levels.

The above studies show that the animal has a very limited ability to store amino acids as such. This limitation is apparent whether amino acids as such, or proteins deficient in one or more amino acids, are fed. The supplementary action of proteins is apparent only when the proteins are fed simultaneously.

The work of van Dam-Bakker and coworkers (*loc. cit.*) indicates that the reproductive capacity of an animal is more resistant to certain dietary disturbances than is growth. However, lactational difficulties may become apparent under the above conditions. Their work suggests that when animals are raised on a poor diet through weaning, they are unable to recover completely from the resulting set-back even though fed thereafter a good ration. This observation deserves further study, since it implies that if nutritional insults are imposed on very young animals, they may never completely recover.

PITUITARY FUNCTION IN HYPOTHYROIDISM

Normal growth in man, as well as in most mammals, depends on the presence of both the thyroid hormone and the pituitary growth hormone. The absence of thyroid function in the human infant leads to the condition called cretinism which is characterized by a great retardation of the rate of growth and the maturation of cartilage centers of ossification. The treatment of such individuals leads to the prompt resumption of growth and maturation.

The involvement of the thyroid in the growth process has been the subject of much investigation. It can be shown in experimental animals after hypophysectomy that thyroid hormone in itself causes some stimulation of epiphyseal growth. Moreover, thyroid hormone greatly augments the action of growth hormone. It is clear, therefore, that the thyroid hormone acts directly at the tissue level to promote the growth processes.

Evidence has accumulated that thyroid deficiency may also influence growth by disturbing pituitary function. A. N. Contopoulos, M. E. Simpson and A. A. Koneff (*Endocrinol.* **63,** *642 (1958)*), have presented particularly pertinent observations bearing on this point. Rats were subjected to thyroidectomy and subsequently the concentrations of thyrotropic, gonadotropic and growth hormones were estimated in the plasma and pituitaries of these animals by injecting these materials into hypophysectomized rats. At both 21 and 56 days after thyroidectomy the plasma of rats contained increased concentrations of thyroid stimulating activity but decreased concentrations of growth hormone as measured by the width of the tibial epiphyses in the assay animal. No increase in gonadotropin concentration of plasma could be demonstrated. The pituitary gland gave evidence of a reduced content of all the hormones studied.

The results with thyroidectomized rats were compared with those found with gonadectomized male rats. The plasma of these latter animals contained an increase in gonadotropic activity but no change in growth promoting activity. The gonadotropic activity of the pituitaries of castrated rats was increased but normal amounts of growth and

thyrotropic activities were found. In further experiments in which rats were both castrated and thyroidectomized, both gonadotropic and thyrotropic activities of serum were augmented. Again, however, the growth promoting activity of such serum was decreased.

It would appear from these observations and other results in the literature that the pituitary content and the rate of secretion of growth hormone is decreased in the hypothyroid state. Examination of the pituitaries histologically provided additional evidence suggesting decreased growth hormone levels in that the acidophils underwent extreme degranulation and became difficult to distinguish from the chromophobe cells. As described by H. D. Purves and W. E. Griesbach (*Endocrinol.* **49,** *244* (*1951*); **49,** *652* (*1951*)) a specific type of basophil recognizable by location and cytologic characteristics undergoes remarkable changes after thyroidectomy and eventually becomes greatly distended with vacuoles. The ultra structure of these thyroidectomy cells has been carefully described by M. G. Farquhar and J. F. Rinehart (*Ibid.* **55,** *857* (*1954*)).

A specific change in the electrophoretic distribution of pituitary proteins has been found to occur after thyroidectomy (H. A. Levey and S. Roberts, *Endocrinol.* **63,** *629* (*1958*)). These authors homogenized rat pituitary glands in saline and obtained a supernatant fraction after centrifugation at 15,000 times gravity. Electrophoresis was carried out on paper in a diethylbarbiturate buffer at pH 8.6. Extracts from anterior pituitaries of rats, rabbits, guinea pigs, dog and cattle possessed a dense protein band which migrated toward the anode with a mobility similar to beta globulin of human serum. Great attenuation of this band occurred after thyroidectomy but not after castration or adrenalectomy. Treatment of the hypothyroid rat with thyroxine restored the missing band to the electrophoresis. In the presence of serum the preferential incorporation of lysine-2-C^{14} by pituitaries into the area of the dense band could be demonstrated after incubation, but after thyroidectomy when the band was greatly decreased the incorporation of lysine into the proteins migrating in this region was unimpaired. This suggested to the authors that the amino acid had entered minor protein constituents with mobilities similar to the "thyroid-labile" component. It was further shown that growth hormone and thyrotropin migrate in this area of the electrophoresis, but evidence is cited that these hormones could not account for the observed behavior of the "thyroid-labile" component. On the basis of such indirect evidence the "thyroid-labile" component was associated with material identical with or derived from, acidophilic granules.

Studies of respiration of rat pituitaries by H. A. Levey and S. Roberts (*Am. J. Physiol.* **189,** *86* (*1957*)) have also demonstrated significant changes after thyroidectomy. After thyroidectomy or prolonged administration of thiouracil drugs, pituitary hypertrophy and hyperemia occurred. This was associated with an increase in oxygen consumption of the pituitaries as measured in the Warburg apparatus. Of course, nearly all other body tissues show a decreased respiratory rate in thyroid deficiency. The increase in anterior pituitary respiration was not accompanied by an accelerated uptake of glucose or production of lactate, and there was no alteration in the level of succinic dehydrogenase or cytochrome oxidase activity of homogenates of the pituitaries. It is important that there was not a comparable increase in the respiration in pituitaries which had become hyperplastic after gonadectomy. Also, the changes which follow hypothyroidism can be reversed by thyroxine administration.

It can be said, in summary, that profound alterations occur in the anterior pituitary gland following thyroidectomy, which include modification of the fine and ultra structure of the thyrotropic and acidophilic

cells, changes in hormone content and secretory rates, and changes in respiratory rate and electrophoretic protein distribution. In the rat, hypothyroidism induces not only overactivity of the thyrotrophic basophils but results in a decrease in growth hormone production. It is reasonable to consider the decrease in growth hormone production as contributing significantly to the growth failure of the thyroprivic state.

SUPPLEMENTATION OF INEFFICIENT PROTEIN

It is generally recognized that one of the most common and most insidious dietary deficiencies of the present day world is that of complete protein. Although some of the various forms which this deficiency disease may take have been recognized and properly ascribed to the correct cause only relatively recently, great progress has been made in diagnosis and treatment, at least in communities with adequate facilities. While means of prevention of the disease are known, in actual practice this has not been simple.

The basic cause of the most serious form of the disease appears to be too early weaning to a diet which is inadequate with respect to complete protein. Correction could, of course, be achieved by a larger proportion of meat products or milk in the diet. These substances, however, may be unavailable or too expensive in those areas in which protein deficiency is common.

Cultivation of different plant foods, some of which may furnish adequate protein by themselves, others of which may supplement each other, may be urged (*Nutrition Reviews* **14,** *291* (*1956*); **15,** *169* (*1957*)). This method may fail when it is contrary to the agricultural or dietary habits of the population. Fish and fish products are rich sources of proteins but may be readily available only in limited areas (*Ibid.* **12,** *72* (*1954*)).

Another possibility, particularly in those countries in which bread is, or can become, a staple food, is the supplementation of the diet with specific amino acids which may be lacking in the native foods. Some success has been reported for supplementation of infants' diets with lysine, the amino acid most likely to be limiting on the vegetarian diet (*Nutrition Reviews* **14,** *101* (*1956*)).

N. W. Flodin (*Am. J. Pub. Health* **48,** *1315* (*1958*)) and his co-workers have carried out many experiments testing the effects of lysine supplementation of otherwise low-efficiency proteins. Growth was retarded when rats were maintained on diets adequate in all respects except that the protein, which was only 12 per cent of the diet, consisted of 85 per cent wheat protein and 15 per cent non-fat milk solids. The males in particular exhibited the symptoms of irritability, anorexia, perverted appetite and decreased longevity. The females had less severe symptoms, slowly approached normal size and bore litters which were, however, subnormal. Symptoms of special significance for human studies were a reduced level of voluntary action and a low caloric intake.

Protein efficiency could be increased in three principal ways; by supplementation with a second protein which might furnish the amino acids lacking in the first, by direct addition of the amino acids in question, or by a combination of both methods. It was found by Flodin and his associates that the protein efficiency (grams weight gained by animals divided by the grams of protein consumed) could be increased by 50 to 80 per cent by increasing the proportion of milk proteins to 30 to 40 per cent of the total. A similar increase could be obtained with 0.25 per cent lysine monohydrochloride. A combination of the two supplements however, increased protein efficiency by 150 per cent, an increase greater than could be achieved by either supplement alone. All three types

of supplementation relieved the deficiency symptoms in the rats.

The author states that some of the symptoms of protein deficiency in human children are similar to those in rats. In particular, the apathy and inactivity of the children and a similar, though milder condition in adults leads to a vicious circle (poor diet → low work output → low food production → poor diet). Supplementation of flour with lysine may seem uneconomical at first. However, it has been found that, at least in rats, 3 per cent nonfat dry milk plus 0.25 per cent L-lysine monohydrochloride is equivalent in growth promotion to 12 per cent nonfat dry milk as a flour additive (J. K. Jahnke and C. Shuck, *J. Nutrition* **61,** *307 (1957)*). The only economic requirement, therefore is to produce lysine at about $\frac{1}{36}$ the cost of the milk powder, an achievement which Flodin states is easily forseeable. However non-fat dry milk furnishes a generous source of other nutrients such as riboflavin and calcium in addition to lysine and other amino acids.

With several methods presently available for increasing protein efficiency, it should be possible, in any specific area, to initiate a program of protein supplementation which would conflict minimally with existing dietary habits and economic status.

UTILIZATION OF PROTEINS

Several investigators have demonstrated a decrease in the biological value of protein following treatment with severe heat. However, only a few studies of this phenomenon have been carried out on the proteins of muscle. A. F. Morgan and G. E. Kern (*J. Nutrition* **7,** *367 (1934)*) reported a progressive decrease of 5 to 30 per cent in utilizability of beef and horsemeat when this was boiled or autoclaved. W. H. Seegers and H. A. Mattill (*J. Nutrition* **10,** *271 (1935)*) found similar effects from heating or hot alcohol extraction of beef liver, kidney, heart or round. Further digestibility studies led these authors to conclude that the low biological value of alcohol-extracted or heated liver was due solely to lowered digestibility, the resulting proportions of amino acids not being representative of the original protein. C. E. Poling, H. W. Schultz and H. E. Robinson (*J. Nutrition* **27,** *231 (1944)*) found the nutritive value of the proteins of cured pork shoulder slightly, but significantly, lowered by a commercial canning process.

P. Wheeler and A. F. Morgan (*J. Nutrition* **64,** *137 (1958)*) have also been concerned with the possibility of decreased absorption of amino acids from the gut following heat treatment of the protein. They reason that a clear elucidation of this possibility may be obtained by comparing rates of digestion of raw and overheated proteins *in vivo* as indicated by assays of amino acids in portal blood after feeding.

In their experiments meat of raw fresh hams was freed as completely as possible from fat, it was ground thoroughly and portions of it were autoclaved at 120°C. for 15, 30, 60, 120, 195 or 240 minutes. In one series, male rats were divided at weaning into groups of seven each. They were fed three times a week one third of a weighed amount of meat raw or autoclaved for 15, 30, 60, 120, or 195 minutes which was calculated to supply them with 5 g. of protein per week. A non-protein basal diet and water were provided *ad libitum*. After four weeks, the rats were fasted of protein for 48 hours, but allowed free access to the non-protein basal diet and water. On the third day four rats each from the groups which had received the raw pork and that autoclaved up to 195 minutes were bled as fasting control animals. The other three rats in each group were fed 5 g. each of the meat which they had been fed previously and were bled one half hour after this meal.

Other rats were maintained on a satisfactory stock diet until their body weight

reached 250 to 350 g. in the males. They were offered 10 g. each of either raw fresh pork or fresh pork autoclaved at 120°C. for four hours. The next day they were given 5 g. of the same meat and portal blood was then taken at predetermined time intervals.

Amino acids of portal blood plasma were determined by quantitative paper chromatography. All amino acids examined in the portal blood plasma were found to be present in much greater concentrations in rats fed raw meat. The increases in the young rats fed raw pork were from 101 to 340 per cent, and in those fed autoclaved pork from −33 to 95 per cent. In the adult rats, the rises were from 48 to 294 per cent when raw meat was given as contrasted to −4 to 88 per cent in those fed autoclaved meat.

Comparison of the differences in plasma concentrations of amino acids as measured *in vivo* with amino acids released *in vitro* (J. F. Benk, F. W. Chornock and E. E. Rice, *J. Biol. Chem.* **180,** *1243 (1949)*) indicates that the differences encountered *in vivo* are much greater than those *in vitro*. Threonine, for example, was artificially liberated from pork autoclaved for four hours in 57.5 per cent as great a quantity as from the raw meat, whereas the percentage increases of threonine in the portal blood plasma of animals fed autoclaved pork were only 27, 44 and 23 per cent of those following the ingestion of raw meat by immature males, adult males and adult females, respectively.

In all, Wheeler and Morgan determined the concentrations of 15 different amino acids in the portal plasma after feeding. The highest values of the absorption curve were at three-fourths hour after feeding in the adult males and, except for glycine, at one-half hour in the adult females. More variability in amino acid concentrations were observed after feeding autoclaved pork than with raw pork.

These workers are of the opinion that the variability in time of peak values for the amino acids in plasma from portal blood during digestion of autoclaved pork, along with the consistently lower percentage increases at the same time in the rats fed autoclaved meat, are facts that support the theory that overheating of pork protein results in a decrease in the digestibility of the protein. Digestive enzymes are apparently incapable of hydrolyzing properly the overheated proteins so as to make available to the animal simultaneously an assortment of amino acids favorable to good growth or maintenance.

CARBOHYDRATE AND PROTEIN ABSORPTION

The rate of protein absorption from the intestinal tract has not been extensively investigated, partly because the mixing of dietary protein with endogenous protein of the digestive secretions makes interpretation of the data difficult. (For a review of some work on this see *Nutrition Reviews* **16,** *334 (1958)*).

Workers at the University of Rochester, Rochester, New York, have reported gastric emptying and intestinal absorption of carbohydrate and protein as influenced by two carbohydrate sources (banana and dextrin) and high (48 per cent) and low (16 or 0 per cent) casein concentrations in the diet (S. Rosenthal and E. S. Nasset, *J. Nutrition* **66,** *91 (1958)*).

Adult male albino rats, weighing 175 to 260 g., were adapted to the various diets for five days prior to the experiments. After a 48-hour fast, 6.2 ml. diet suspension (1.5 g. of dry diet) were introduced into the stomach by means of a catheter. Identical samples were analyzed chemically for total carbohydrate and total protein (N × 6.25).

At hourly intervals (one through six hours) gastric and intestinal contents of the test animals were obtained after sodium pentobarbital anesthesia and appropriate ligations. The contents of the stomach and

gut were diluted to 250 and 500 ml., respectively, homogenized, and enzyme-inactivated prior to analysis.

Protein in the diet had no influence on carbohydrate absorption or stomach-emptying time. There was, however, a significant effect of carbohydrate source. The time required to empty 50 per cent of the banana carbohydrate from the stomach, derived from equations for the regressions (Log. per cent gastric carbohydrate plotted against time), was 107 minutes, contrasted with 46 minutes for the dextrin. Similarly, the absorption of the dextrin occurred at an accelerated rate when compared with the banana carbohydrate. Sixty per cent of the dextrin in the test meal was absorbed in two hours while three hours were required for comparable absorption of banana carbohydrate. Per cent absorption was derived from the amount ingested minus the amount found in the gut.

The authors noted that the carbohydrates of fresh bananas formed a colloidal mass which at pH 7.0 and 24° C. was 2.5 times as viscous as that which was formed from dextrin. Whether or not this was a factor responsible for the slower rate of absorption was not established.

The most striking feature of the data on protein was the relatively constant load of protein in the small intestine, regardless of level of dietary protein, carbohydrate source, or time after feeding. The average protein recovered from the gut was 119, 127, and 123 mg. for low, intermediate, and high dietary protein levels, respectively. The average hourly recoveries of protein from the gut over the six-hour period were 125, 117, 119, 140, 115, and 118 mg., respectively.

It is evident that the small intestine maintains a protein content in its lumen which is not greatly changed by the amount of protein in the diet. This is consistent with the studies of others who reported that the water content and the total dry matter content of the intestine remain constant over a postprandial period of nine hours (S. Lepkovsky, R. L. Lyman, D. Fleming, M. Magumo, and M. M Dimick, *Am. J. Physiol.* **188,** *327 (1957)*).

In the studies by Rosenthal and Nasset (*loc. cit.*) it was found that both dietary carbohydrate and protein in the gastric contents diminished exponentially with time, a confirmation of the observations of others working with carbohydrates (P. F. Fenton, *Am. J. Physiol.* **144,** *609 (1945)*; P. C. Reynell and G. H. Spray, *Ibid.* **131,** *452 (1956)*).

The classical determination of "digestibility" of protein involves subtraction of fecal protein (N × 6.25) from food protein and expressing the remainder as a percentage of the protein fed. The basic assumption is made that the fecal protein is composed of undigested food protein. This has been recognized as a fallacy for many years, yet the term "digestibility" is still used in its literal sense.

Based on their own studies and those of others, Rosenthal and Nasset estimate that protein contributed by digestive secretions (stomach, pancreas, and small intestine) could be assumed to be as little as 22 mg. or as much as 94 mg. It is evident that of the hourly intestinal recoveries of protein a considerable portion obviously represented protein derived from these endogenous sources.

Earlier studies by the Rochester workers have yielded evidence to support this view (E. S. Nasset, P. Schwartz, and H. V. Weiss, *J. Nutrition* **56,** *83 (1955)*; E. S. Nasset, *J. Am. Med. Assn.* **164,** *172 (1957)*). It is worthy of mention that Geiger and associates, (E. Geiger, L. E. Human and M. J. Middleton, *Proc. Soc. Exp. Biol. & Med.* **97,** *232 (1958)*), based on their data with rats, questioned that there was much dilution of food protein with digestive protein in the intestine. All of their studies, however, were limited to a single absorption period of three hours.

It is evident that this problem would be considerably clarified by feeding a labeled protein so that a direct determination could be made of its dilution with endogenous protein at various times after feeding.

NOTES

Sir:

In a review entitled, "In Vivo Protein Digestion" (*Nutrition Reviews* **16,** *334 (1958)*) one of our early papers on this subject (Dreisbach and Nasset, *J. Nutrition* **53,** *523 (1954)*) was mentioned, but later publications were overlooked. Some of the questions brought out by the reviewer have been considered, and at least partially answered, in our later papers.

The digestibility of protein and its nutritive value, for example, have often been assumed to be related to the amino acid mixture present in intestinal contents, but at least in short term experiments it has been shown that in dog and man the mixture of endogenous and exogenous proteins in the digestive tract tends to yield an amino acid mixture of relatively constant composition (Nasset and Davenport, *J. Applied Physiology* **7,** *447 (1955);* Nasset, Schwartz and Weiss, *J. Nutrition* **56,** *83 (1955)*; Nasset, *J. Am. Med. Assn.* **164,** *172 (1957)*).

The magnitude of the endogenous contribution of protein during normal digestion cannot be estimated by feeding a non-protein test meal, as the reviewer suggests, because it fails to provide a maximal stimulus for digestive secretions. The magnitude of the endogenous protein contribution to intestinal contents, from secretions and sloughed mucosa, is unexpectedly high as was confirmed by feeding radioactive casein to dogs and rats (Nasset and Ju, *Fed. Proc.* **17,** *486 (1958)*)

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Vitamin B_{12} Absorption in Carriers of *Diphyllobothrium latum*

A study of the absorption of vitamin B_{12} was conducted on 50 non-anemic carriers of *D. latum*, seven carriers of this parasite with megaloblastic anemia and 50 healthy control subjects using the Schilling test in the following manner: "The fasting subjects were given 0.5 to 2.0 micrograms of cobalt^{60}-labeled vitamin B_{12} (containing about 160 microcuries/milligram) in 200 ml. pH 7.4 phosphate buffer. Ninety minutes later, 1 mg. of non-labeled vitamin B_{12} was injected intramuscularly. A twenty-four hour collection of urine was started on the beginning of the test; the vessel used contained non-labeled vitamin B_{12} as a carrier" (*New Engl. J. Med.* **29,** *216 (1958)*).

The results of this test showed mean urinary excretion values of 15.91 and 15.82 per cent of the administered dose for the control group and 0.9 per cent for the patients with pernicious anemia. All of the parasite carriers with megaloblastic anemia had excretion results which were definitely in the pernicious anemia range. Only nine non-anemic worm carriers had excretion values which were definitely within normal range. After the expulsion of the worm, three of the anemic and three of the non-anemic patients had labeled vitamin B_{12} excretion values within the pernicious anemia range. On retesting four of these at a later date, two had persistently low excretion values and could be regarded as cases of true pernicious anemia. It is of interest to note that even the parasitized patients with normal excretion values showed a definite rise in these values after expulsion of the worm. Previous investigations along the same line, but in the course of which fecal excretion of labeled vitamin B_{12} was measured (*Acta Haemat.* **19,** *90 (1958)*), had also shown that the presence of *D. latum* prevented optimal absorption of the vitamin even in carriers who had absorption values within normal range.

The exact mechanisms through which this worm inhibits vitamin B_{12} absorption are not clear. The possibilities which are considered are that the worm may break down the interaction product of vitamin B_{12} and

intrinsic factor, impair the ability of the intestinal wall to absorb vitamin B_{12}, or the worm itself may take up this vitamin or the bound vitamin intrinsic factor product.

It must be mentioned here that the above finding of the effects of the presence of *D. latum* on the absorption of vitamin B_{12} by the carrier host does not apply to all species of *D. latum* (*New Engl. J. Med.* **253,** *808 (1955)*), the species in the studies described being of North European distribution.

Bananas in Low-Sodium Regimens

Patients affected with conditions necessitating a drastic reduction in sodium intake have become confronted with the prospect of living on a very monotonous and unpalatable diet, such as the rice diet, for undetermined periods of time. Palatable supplements, which could vary the menu without altering its sodium content, would be of great use in helping the patient persevere with such a rigid dietary regimen essential to his well-being.

The banana, which has previously been mentioned to have a low sodium content, seems to fit such requirements and has become the object of a study along this line (*New Engl. J. Med.* **259,** *901 (1958)*). Sodium and potassium contents of the banana were determined, taking into account the following possible modifying factors; soil type and duration of cultivation, rainfall, fertilizers, and location of the individual banana on the tree and in the cluster, as well as the location on the fruit of the sample section on which the determinations were made. The Gros Michel variety of bananas, the variety most widely imported in the United States, was used for this study.

With regard to the section of the individual fruit or the position of the individual banana in the bunch, the differences in sodium content were of no practical significance, all of them being less than 1 microgram per gram of the fruit. The amount of rainfall or length of cultivation of the land likewise did not cause any major variation. Heavy fertilization, however, increased the sodium content at least four times. Since this factor occurred only under special cultivation experiments, it does not assume practical importance, since the products of such farms would not be distributed widely commercially. None of the factors which have been mentioned as possible sources of variation affected the potassium content of bananas, the mean value being 3.76 ± 0.29 mg. per gram of fruit.

The average 100 g. banana provides 100 calories and is high in vitamin content. If ten bananas a day are used in the diet, they would provide only 0.02 mEq. of sodium. This, in addition to low-sodium milk which is now available, would make the low-sodium diet more practical, more palatable and more acceptable to patients.

Two cases illustrate this study, one of anasarca due to congestive heart failure and the other of liver cirrhosis with ascites. Both responded with profound diuresis after having been placed on the banana and low-sodium milk regimen.

Recent Books

Nutrition in Health & Disease. L. F. Cooper, E. M. Barber, H. S. Mitchell. Published by J. B. Lippincott Co., Philadelphia. Pp. 734. Price $6.00.

Trends in Genetic Analysis. G. Pontecorvo, Fellow of the Royal Society, Professor of Genetics, University of Glasgow. Published by Columbia University Press, New York. Pp. 145. Price $4.00.

Food for Better Performance. R. C. Hutchinson. Published by Melbourne University Press, New York; Cambridge University Press. Pp. 98. Price $2.75.

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